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Center for Veterinary Biologics
Testing Protocol

SAM 510

Supplemental Assay Method for the Manual Determination
of Formaldehyde in Veterinary Biologics (Schiff Test)

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1. Introduction

This Supplemental Assay Method (SAM) may be used for the colorimetric determination of free formaldehyde in killed viral and bacterial products. The test is based on the classical Schiff's reaction for determining aldehydes.

2. Materials

2.1 Equipment/instrumentation

Brand names are provided for reference only, equivalent equipment or materials may be used.

2.1.1 Spectrophotometer or colorimeter with cuvettes (Bausch and Lomb Spectronic 70)

2.1.2 Centrifuge capable of 800-1000 x G.

2.1.3 Routine laboratory glassware and supplies, including pipets, screw cap test tubes, conical screw cap centrifuge tubes, test tube racks, class A volumetric flasks, laboratory toweling or wipes, laboratory timer, beakers, pipettor bulb (repipettors are optional), graph paper, and adjustable ship curve

2.2 Reagents/supplies

All chemicals are reagent grade unless specified. Store at room temperature, unless otherwise specified. All chemicals and solutions are good for 1 year, unless specified.

2.2.1 Dilute hydrochloric acid, 2.5% (NVSL Media 30036, specify formula and volume, Appendix I)

2.2.2 Formaldehyde Solution, 37% (Formalin)--Check formaldehyde content of reagent by USP method (Reference 7.2). Prepare 1% standard formalin solution by diluting 1 ml of 37% formaldehyde to 100 ml with water (use 100-ml volumetric flask).

2.2.3 Modified Schiff Reagent, (NVSL Media 30019, Appendix II) This reagent remains satisfactory for use for several weeks, as long as it retains a strong odor of sulfur dioxide.

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2.2.4 Water--Use deionized or distilled water or water of equivalent purity.

2.2.5 Emulsified samples (oil and water emulsion) require that the emulsion be broken before the test is run. Chemicals needed for this step:

1. Sodium chloride, 10%--(NVSL Media 30192, specify formula and volume, Appendix III)
2. Chloroform

3. Preparation for the test

3.1 Personnel qualifications/training

No specific training is required. Individual should have working knowledge of laboratory equipment listed in **Section 2**.

3.2 Preparation of equipment/instrumentation

Turn on spectrophotometer to allow the instrument to "warm up" for at least 30 minutes.

3.3 Preparation of reagents/control procedures

Prepare a 1% formalin solution by diluting 1 ml of 37% formaldehyde with water to 100 ml in volumetric flask. Use this solution as a control, diluting it as the standard solution and testing it at 2 levels (run 0.2% and 0.4% or levels that bracket the samples).

3.4 Preparation of the sample

Reference the current version of TCSOP0001 for sample submission.

Critical control point: Dilute the formalin standards and all samples to a constant ratio so that standards and samples are treated the same. The colorimetric reaction is very dependent upon time and temperature. The reaction is carried out at room temperature, consequently, it may be necessary to adjust the dilution to fit the conditions in each laboratory.

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3.4.1 Dilute aqueous samples 1+3 (1+2 to 1+5) with water. Make duplicate dilutions.

3.4.2 Emulsified samples require that the emulsion be broken by adding 2 ml of sample to 4 ml of 10% NaCl and 6 ml of chloroform. This mixture is shaken for 30 seconds and then centrifuged for 5 minutes at 800-1000 x G. The clear upper layer is used in **4.1.3**. A separate standard curve should be prepared for these products in which the standards are treated the same as the emulsified samples.

4. Performance of the test

4.1 Samples

4.1.1 For each sample dilution, pipet 10 ml of 2.5% HCl into a tube and mark it (sample No.____).

4.1.2 Also for each sample dilution, pipet 12 ml of 2.5% HCl into a tube and mark it (sample blank No.____).

4.1.3 Place 1 ml of diluted sample into each of the tubes (**4.1.1** and **4.1.2**) and mix. Remember to make dilutions.

4.2 Standards

4.2.1 Pipet 2.5% HCl into a tube and mark the tube (standard blank). Use this tube to set the instrument at zero O.D.

4.2.2 Prepare tubes containing the 2.5% HCl and the diluted standard as described in the following table. Mark the tubes indicating the appropriate concentration of the standard.

Formalin equivalent(%)	0.1	0.2	0.3	0.4	0.5	0.6	0.7
ml of 2.5% HCl	10.9	10.8	10.7	10.6	10.5	10.4	10.3
ml of standard	0.1	0.2	0.3	0.4	0.5	0.6	0.7

4.2.3 Mix by gently inverting the tube.

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4.3 In sequence, add 2 ml of Schiff Reagent to each tube marked sample or standard at 1 minute intervals. Do not add Schiff Reagent to any "blank" tubes. All tubes, at this point, should contain 13 ml.

4.4 Mix the tubes well but keep them in the proper sequence (mix immediately after the reagent is added).

4.5 Allow the color to develop for exactly 30 minutes from the time the first Schiff Reagent was added. Read O.D. at 570 nm and record the O.D. for each tube in sequence at 1 minute intervals.

4.6 Read and record the O.D. of each sample blank.

5. Interpretation of the test results

5.1 Draw a curve by plotting the O.D. of the standards versus the percent formalin.

5.2 Calculations

5.2.1 Subtract the O.D. of the sample blank from the O.D. of the sample. Record this difference.

5.2.2 Determine the percent formalin in the sample by comparing the net O.D. of the sample to the standard curve.

5.3 Retest

5.3.1 If the O.D. of the sample reads outside the end points of the standard curve, redilute the sample and retest (multiply results from the curve by this dilution factor).

5.3.2 If the color developed is extremely dark, redilute and retest. (If the color is faint, test at a lower dilution or check reagent, reagent may need to be replaced).

5.3.3 If the controls vary more than 10% from the expected value, make new standard and control solutions, redilute the sample, and retest.

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6. Report of test results

Test results are reported following the current version of TCSOP0001.

7. References

7.1 Reference is made to the long term use of this procedure by the Toxicology and Chemistry Section.

7.2 U.S. Pharmacopia/National Formulary, current issue.

8. Summary of revisions

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the name of the contact person has changed.

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9. Appendices

Appendix I

NVSL media 30036

Author's Comment: Media 30036 is generic and open-ended for HCl solutions. The requestor must request volume needed as well as the formulation. In the case of this protocol, specifics are:

Prepare 1 liter by slowly adding 25 ml concentrated HCl to approximately 500 ml water in a 1-liter volumetric flask. Fill to volume.

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Appendix II

NVSL media 30019

(Retyped here to fit the space)

SCHIFFS REAGENT, MODIFIED (STERILITY)

DISSOLVE 0.05g OF BASIC FUCHSIN IN 90ML OF DISTILLED
WATER. THEN ADD 1g OF SODIUM SULFITE, MIX UNTIL
COMPLETELY DISSOLVED AND THEN ADD 1ML OF CONCENTRATED
HCL AND DILUTE TO ONE LITER WITH WATER. KEEP SOLUTION
IN WELL-STOPPERED AMBER BOTTLE.

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Appendix III

NVSL media 30192

Author's Comment: Media 30192 is generic and open-ended for NaCl solutions. The requestor must request volume needed as well as the formulation. In the case of this protocol, specifics are:

Prepare 100 ml by adding 10 g NaCl to approximately 50 ml water in a 100-ml volumetric flask. Dissolve and fill to volume.